TRADE SECRET

Study Title

H-28072:
Bacterial Reverse Mutation Test

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.5100 (1998)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 471) (1998)

EC Commission Directive 2000/32/EC Annex 4D–B.13/14
Number L 136

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LABORATORY PROJECT ID: DuPont-22734

WORK REQUEST NUMBER: 17199

SERVICE CODE NUMBER: 500

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the items documented below. None of the items listed impact the validity of the study.

1. The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

2. Neither the vehicle nor the positive controls were characterized by the testing facility or the sponsor. However, both the vehicle and positive controls were purchased from a reputable vendor and showed results consistent with historical control data.

3. The concentrations of the positive control and test substance dose solutions were not confirmed analytically; however, the solutions were prepared by trained personnel to ensure the accuracy of the concentrations.

Study Director: E. Maria Donner 13-AUG-2008

E. Maria Donner, Ph.D. Date
Senior Research Toxicologist and Manager

QUALITY ASSURANCE STATEMENT

Work Request Number: 17199
Service Code Number: 500

Key inspections for DuPont work request 17199, service code 500 were completed by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	March 27, 2007	March 27, 2007	March 27, 2007
Conduct:	April 3, 2007	April 3, 2007	April 3, 2007
Report/Records:	June 12, 2007 July 16, 2007 July 25, 2007	June 12, 2007 July 16, 2007 July 24, 2007	June 18, 2007 July 16, 2007 July 24, 2007
Report Revision 1:	August 12, 2008	August 12, 2008	August 12, 2008

Reported by: Donna M. Johnston — 12 Aug 2008
Donna M. Johnston, Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed and Approved by: Jeff E Jowell for SRF — 12 AUG 2008

Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P.
Research Fellow and Manager — Date

Issued by Study Director: E Maria Donner — 13-AUG-2008

E. Maria Donner, Ph.D.
Senior Research Toxicologist and Manager — Date

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STUDY INFORMATION

Substance Tested:
- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28072

Haskell Number: 28072

Composition:

82.6%	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionate*
13.9%	Water
3.5%	Ammonium
0.41%	Organic Impurities

* Note: The Ammonium-2,3,3,3-tetrafuoro-2-(heptafluoropropoxy) propionate component (HFPO Dimer ammonium salt) contains 0.1 ppm HFPO trimer ammonium salt.

Purity: See composition, above

Physical Characteristics: Clear and colorless concentrated aqueous solution

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: March 21, 2007 / (see report cover page)

Experimental Start/Termination: March 27, 2007 / April 5, 2007

REASON FOR REVISION 1

At the request of the sponsor, the units for dosing concentration were standardized throughout the Material and Methods and Results and Discussion sections.

SUMMARY

The test substance, H-28072, was evaluated for mutagenicity in the Bacterial Reverse Mutation Test using the plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvr*A were tested in the absence and presence of an exogenous metabolic activation system (Aroclor-induced rat liver S9).

The test was performed in 2 phases. The first phase was the toxicity-mutation test which established the dose range for the mutagenicity test, and provided a preliminary mutagenicity evaluation. The second phase was the mutagenicity test which evaluated and confirmed the mutagenic potential of the test substance.

Sterile water was chosen as the dosing vehicle based on the solubility of the test substance and compatibility with the target cells. The test substance formed a clear and soluble solution in water 50 mg/mL, the highest concentration that was tested in the study.

In the toxicity-mutation test, the maximum dose evaluated was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the absence and presence of S9 metabolic activation. Due to the discrepancy between the initial sponsor-reported and COA-reported purities, the actual maximum exposure concentration tested was 4885 µg/plate. The lower concentrations were consistently reduced by 2.3% each. All subsequent exposure concentration levels in this report represent the nominal values based in the initial sponsor-reported purity (84.5%) used for study conduct. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg/plate. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level in any tester strain in the absence or presence of S9 metabolic activation. A >50% reduction in mean number of revertants was observed at 100 µg/plate and 5000 µg/plate for TA1537 without S9 activation; however, this reduction occurred with no dose related correlation. No toxicity was observed at any other dose level with any other tester strain in either the absence or presence of S9 activation. No test substance precipitation was observed at any dose level with any tester strain in either the absence or presence of S9 metabolic activation.

Based on the toxicity-mutation test, the maximum dose evaluated in the mutagenicity test was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the absence and presence of S9 metabolic activation. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 333, 667, 1000, 3333, and 5000 µg/plate for all tester strains. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level or with any tester strain in either the absence or presence of S9 metabolic activation. No toxicity or test substance

precipitation was observed at any dose level with any tester strain in either the absence or presence of S9 metabolic activation.

All criteria for a valid study were met. Under the conditions of this study, H-28072 showed no evidence of mutagenicity in the Bacterial Reverse Mutation Test either in the absence or presence of Aroclor-induced rat liver S9. It was concluded that the test substance was negative in this *in vitro* test.

INTRODUCTION

The objective of this study was to evaluate the test substance, H-28072, for its ability to induce reverse mutations at the histidine locus in the genome of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and at the tryptophan locus of the *Escherichia coli* strain WP2*uvr*A. The assay was conducted with and without an exogenous S9 metabolic activation system.

MATERIALS AND METHODS

A. Test Guidelines

Except as noted below, the study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.5100: Bacterial Reverse Mutation Test, *Health Effects Test Guidelines* (1998)

- Ninth Addendum to the OECD, Section 4 (Part 471): Bacterial Reverse Mutation Test, *Guideline for the Testing of Chemicals* (1998)

- European Commission Directive 2000/32/EC of May 19, 2000, Annex 4D-B13/14. Mutagenicity - Reverse Mutation Test Bacteria. Number L 136

– The initial sponsor-reported purity for H-28072 was 84.5% active ingredient. A correction factor of 1.183 was used for preparation of the dosing solutions. However, the COA that was issued after the experimental termination of the study reported a purity of 82.6%. The guideline recommended limit dose for this test system is 5000 µg/plate. Although the actual maximum dose (4885 µg/plate) did not reach this limit, the difference (2.3%) was considered negligible. This deviation did not impact the validity or outcome of this study.

B. Test Substance and Controls

1. Identification

The test substance, H-28072, was a clear and colorless concentrated aqueous solution. The test substance used for this study was assigned Haskell identification number 28072. Additional information regarding the test substance is located on the study information page of this report.

2. Characterization

The test substance was characterized by the sponsor prior to this study. The Certificate of Analysis (COA) of the test substance is included in this report (Appendix A).

3. Sample Preparation, Stability, and Analytical Verification of Test Substance Concentrations

The initial sponsor-reported purity for H-28072 was 84.5% active ingredient. A correction factor of 1.183 was used for preparation of the dosing solutions. However, the COA that was issued after the experimental termination of the study reported a purity of 82.6%. An analytical verification of the test substance concentrations was not conducted.

4. Controls

Negative:	sterile water (CAS 7732-18-5, molecular grade, Mediatech Inc.)
Positive (Moltox Inc.):	benzo[a]pyrene [CAS 50-32-8] 4-nitroquinoline N-oxide [CAS 56-57-5] acridine mutagen ICR-191 [CAS 17070-45-0] sodium azide [CAS 26628-22-8] 2-aminoanthracene [CAS 613-13-8] 2-nitrofluorene [CAS 607-57-8]

The positive controls were dissolved in DMSO (DMSO, CAS 67-68-6, 99.9% purity, EMD), except for sodium azide and ICR-191, which were dissolved in sterile water. The positive controls appeared to be stable during this test and no evidence of instability was observed.

C. Test System and Test System Justification

The tester strains were the *Salmonella typhimurium* histidine auxotroph tester strains TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* tryptophan auxotroph WP2*uvr*A.[1,2,3] All tester strains were obtained from Moltox Inc. (Boone, NC). These strains were selected due to their ability to revert to histidine and tryptophan independence when exposed to a mutagen. The specific genotype characteristics of these strains are as follows:

		Additional Mutations		
Tester Strain	HIS/Trp Mutation	Repair	LPS	Plasmid
S. typhimurium TA98	*his*D3052	Δ*uvr*B	*rfa*	pkM101
S. typhimurium TA100	*his*G46	Δ*uvr*B	*rfa*	pkM101
S. typhimurium TA1535	*his*G46	Δ*uvr*B	*rfa*	--
S. typhimurium TA1537	*his*C3076	Δ*uvr*B	*rfa*	--
Escherichia coli WP2*uvr*A	*trp*E	Δ*uvr*A	-	--

In addition to a mutation in either the histidine or tryptophan operons, the tester strains contain additional mutations that enhance their sensitivity to some mutagens. A mutation of either the *uvr*A or *uvr*B gene results in a deficient DNA excision repair system. Since the *uvr*B deletion extends through the *bio* gene, the *Salmonella typhimurium* tester strains also require the vitamin biotin for growth.

The *Salmonella typhimurium* tester strains also contain the *rfa* wall mutation which results in the loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide (LPS) barrier that forms the surface of the bacterial cell wall. The resulting cell wall deficiency increases permeability to certain classes of chemicals such as those containing large ring systems that would otherwise be excluded by a normal intact cell wall.

Tester strains TA98 and TA100 also contain the pKM101 plasmid, which further increases the sensitivity of these strains to some mutagens.

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independent (prototrophy) by frameshift mutagens. Tester strain TA100 is reverted by both frame shift and base substitution mutagens. Tester strains TA1535 and WP2*uvr*A are reverted from auxotrophy to prototrophy by base substitution mutagens.

D. Preparation and Storage of Tester Strain

Frozen permanent stocks of all tester strains were prepared by growing fresh cultures and adding 0.09 mL DMSO per milliliter of culture. Aliquots were frozen in dry ice and stored at ≤-70°C.

Master plates were prepared by streaking each tester strain from a frozen permanent stock onto either nutrient agar plates or minimal glucose agar plates. The minimal glucose agar plates were supplemented with either histidine and biotin or tryptophan, and for strains containing the pKM101 plasmid, ampicillin. Tester strain master plates were stored at 5 ± 3°C.

Cultures for use in the study were inoculated from the appropriate master plates. The cultures were placed in a shaker/incubator for overnight at 150 ± 50 rpm and 37 ± 2°C. To ensure that appropriate numbers of bacteria are plated, the length of incubation was determined by spectrophotometric monitoring of culture density.

E. Confirmation of Tester Strain Genotype

Tester strain cultures were checked for the following genetic markers on the day of the preparation of master plates.

The histidine requirement was tested by comparing the growth of each *Salmonella* tester strain on a histidine/biotin-supplemented minimum glucose agar plate with their growth on a biotin-only minimum glucose agar plate.

The tryptophan requirement was tested by comparing the growth of WP2*uvr*A strain on a tryptophan-supplemented minimum glucose agar plate with their growth on a minimum glucose agar plate.

For the *Salmonella* tester strains the presence of the *rfa* wall mutation was confirmed by demonstration of the sensitivity of the cultures to crystal violet.

The presence of *uvr*A and *uvr*B mutation was demonstrated by the sensitivity to ultraviolet light of the tester strains.

The presence of the pKM101 plasmid was confirmed for cultures of tester strains TA98 and TA100 by demonstration of resistance to ampicillin.

F. Experimental Design and Methodology

1. Solubility Determination and Selection of Vehicle

A solubility determination was conducted to determine the maximum soluble concentration or workable suspension up to a maximum of 50 mg/mL. The determination was conducted prior to study initiation or no later than the experimental start date and the data was documented in the study records and final report. Vehicles compatible with this test system, in order of preference, included but were not limited to sterile water (CAS 7732-18-5), DMSO (CAS 67-68-5), ethanol (CAS 64-17-5), and acetone (CAS 67-64-1). The vehicle of choice was the solvent, selected in order of preference, which permitted preparation of the highest workable/soluble stock concentration up to 50 mg/mL.

Based on the solubility of the test substance and compatibility with the target cells, sterile water was chosen as the test substance solvent.

2. Exogenous Metabolic Activation and Sham Mix

Liver homogenate (S9, average protein concentration: 35.8 mg/mL) prepared from male Sprague-Dawley rats induced with Aroclor 1254 was purchased commercially (Moltox Inc., Boone, NC).

The S9 was thawed and the 10% S9 mix prepared immediately prior to its use. The S9 mix was held on ice at all times. The S9 mix contained proportionate volumes of the following components:

Molecular-grade water	2.4 mL
0.825 M KCl/0.2 M $MgCl_2$	0.4 mL
0.2 M phosphate buffer, pH 7.4	5.0 mL
0.25 M glucose-6-phosphate	0.2 mL
0.04 M NADP	1.0 mL
S9	1.0 mL
Total Volume	10 mL

The sham mix was 100 mM phosphate buffer at pH 7.4.

3. Controls

a. Negative Controls

Sterile water, as the negative control, was plated for each tester strain with and without S9 activation.

b. Positive Controls

Tester Strain	S9 Mix	Positive Control	µg per plate
TA98	+	benzo[a]pyrene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	-	Acridine mutagen ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1.0

c. Sterility Controls

One hundred µL of the most concentrated test substance dilution (50 mg/mL), 0.5 mL of S9, and sham mixes were added to selective agar plates to check for sterility.

4. Plate Identification, Frequency, and Route of Administration

Each plate was labeled with the work request number, service code, Haskell number, treatment date, and plate number. The plate number signifies a positive control, a negative control or a sample plate, and tester strain, the absence or presence of S9 metabolic activation, dose level, and replicate.

In the non-activated assays, 0.5 mL of sham mix and 100 µL of vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain.

In the S9-activated assays, 100 µL of the vehicle, test substance dilution, or positive control were added to pre-heated (45–48°C) glass culture tubes containing 2 mL of selective top agar, followed by 100 µL of tester strain and 0.5 mL of S9 mix.

All mixtures were vortexed and overlaid onto the surface of minimum glucose agar plates. After the overlay solidified, the plates were inverted and incubated for approximately 48-50 hours at 37 ± 2°C. Plates that were not evaluated immediately following incubation were stored at 5 ±3°C. All toxicity-mutation test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in duplicate. All mutagenicity test dose preparations of negative (vehicle) controls, test substance, and positive controls were plated in triplicate.

5. Dose Level Determination

The dose levels for the toxicity-mutation test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg/plate. The dose levels for the mutagenicity test were 333, 667, 1000, 3333, and 5000 µg/plate. Due to the discrepancy between the initial sponsor-reported and COA-reported

purities, the actual maximum dose was 4885 µg/plate. The lower dose levels were consequently reduced by 2.3% each. All subsequent dose levels in this report represent the nominal values based on the purity (84.5%) used for study conduct.

6. Toxicity-Mutation Test, Mutagenicity Test, and Test Method

The test substance was evaluated along with negative and positive controls using tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A with and without S9 activation. The plate incorporation method was employed. Dose levels for the mutagenicity test were chosen from the toxicity-mutation test results. The toxicity-mutation test used duplicate plates for each dose level and the mutagenicity test used triplicate plates.

7. Scoring

The appearance of the bacterial background lawn was assessed for test substance toxicity and precipitation. Toxicity was scored relative to the concurrent tester strain specific negative control, and evaluated as a decrease in the mean number of revertant bacterial colonies per plate. In addition, the thinning or disappearance of the bacterial background lawn was considered as signs of toxicity. Precipitation was assessed by visual examination.

Revertant colonies were counted with an automated colony counter (Sorcerer, Perceptive Instruments Ltd., Suffold, United Kingdom). Plates that could not be accurately counted automatically were counted manually.

G. Criteria for Determination of a Valid Test

1. Tester Strain Integrity

To demonstrate the presence of the *rfa* mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the *uvr*A and *uvr*B mutations, all tester strains cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

2. Tester Strain Culture Density

To ensure that appropriate numbers of bacteria are plated, all tester strain culture densities must be approximately 10^9 cells per milliliter.

3. Negative Control Values

The tester strain cultures must exhibit a characteristic mean number of spontaneous revertants per plate when plated along with the negative (vehicle) control under selective conditions. The acceptable ranges for the mean values of negative controls are as follows:

Tester Strain	Negative Control Range
TA98	8-60
TA100	60-240
TA1535	4-45
TA1537	2-25
WP2*uvr*A	5-60

4. Positive Control Values

Each mean positive control value must exhibit at least a 3.0-fold increase over the respective mean negative (vehicle) control value for each tester strain.

5. Toxicity

A minimum of three non-toxic scorable dose levels are required to validate the study. A dose level is considered toxic if it causes:

- A >50% reduction in the mean number of revertants per plate relative to the mean negative control value and exhibits a dose-dependent drop in the revertant count, **or**
- A reduction in the background lawn.

In the event that less than 3 non-toxic dose levels are achieved, the affected portion of the test will be repeated with an appropriate change in dose levels.

6. Data Point Rejection

- A single data point may have been rejected if contamination or excessive toxicity was seen on a treatment plate. A single data point may also have been rejected if excessive precipitate on the plate prevented accurate colony counting.
- A negative control data point may have been rejected if it fell outside the acceptable spontaneous mutation range.
- A positive control data point may have been rejected if it had a low mutagenic response compared to the other positive control plates in that data set.

H. Evaluation of Test Results

Criteria for a positive response:

1. Strains TA1535 and TA1537

Data will be judged positive if the increase in mean revertants at the highest numerical dose response is ≥ 3.0-fold the mean concurrent negative control value (vehicle control). This increase in the mean number of revertants per plate must be accompanied by a dose response

associated with increasing concentrations of the test substance unless observed at the top dose level only.

2. Strains TA98, TA100 and WP2*uvr*A

Data sets will be judged positive if the increase in mean revertants at the highest numerical dose response is ≥ 2.0-fold the mean concurrent negative control value (vehicle control). This increase in the mean number of revertants per plate must be accompanied by a dose response associated with increasing concentrations of the test substance unless observed at the top dose level only.

I. Data Presentation

For each tester strain, the mean of the number of revertants and the standard deviations were calculated.

RESULTS AND DISCUSSION

A. Solubility

The test substance formed a clear and soluble solution in water at 50 mg/mL, the highest stock concentration that was prepared for use on this study. Due to the discrepancy between the initial sponsor-reported and COA-reported purities, the actual stock concentration that was prepared was 48.9 mg/mL.

B. Sterility Controls

No contaminant colonies were observed on the sterility plates for the most concentrated test substance dilution (50 mg/mL) and the S9 and sham mixes.

C. Toxicity-Mutation Test

(Tables 1-10 and 21-22)

In the toxicity-mutation test, the maximum dose evaluated was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the absence and presence of S9 metabolic activation. Due to the discrepancy between the initial sponsor-reported and COA-reported purities, the actual maximum exposure concentration tested was 4885 µg/plate. The lower concentrations were consistently reduced by 2.3% each. All subsequent exposure concentration levels in this report represent the nominal values based in the initial sponsor-reported purity (84.5%) used for study conduct. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 33.3, 66.7, 100, 333, 667, 1000, 3333, and 5000 µg/plate. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level in any tester strain in the absence or presence of S9 metabolic activation. A >50% reduction in mean number of revertants was observed at 100 µg/plate and 5000 µg/plate for TA1537 without S9 activation; however, this reduction occurred with no dose related correlation. No toxicity was observed at any other dose level with any other tester strain in either the absence or presence of S9 activation. No test substance precipitation was observed at any dose level with any tester strain in either the absence or presence of S9 metabolic activation.

D. Mutagenicity Test

(Tables 11-20 and 23-24)

Based on the toxicity-mutation test, the maximum dose evaluated in the mutagenicity test was 5000 µg/plate for tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the absence and presence of S9 metabolic activation. This dose was achieved using a concentration of 50 mg/mL and a 100 µL plating aliquot. The dose levels used in this test were 333, 667, 1000, 3333, and 5000 µg/plate for all tester strains. The plate incorporation method was employed. No positive mutagenic responses were observed at any dose level or with any tester strain in either the absence or presence of S9 metabolic activation. No toxicity or test substance

precipitation was observed at any dose level with any tester strain in either the absence or presence of S9 metabolic activation.

CONCLUSIONS

All criteria for a valid study were met. Under the conditions of this study, H-28072 showed no evidence of mutagenicity in the Bacterial Reverse Mutation Test either in the absence or presence of Aroclor-induced rat liver S9. It was concluded that the test substance was negative in this *in vitro* test.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. Ames, B.N., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Research 31, 347-364.

2. Maron, D.M., and Ames, B. (1983). Revised Methods for the Salmonella Mutagenicity Test. Mutation Research 113, 173-215.

3. Wilcox, P., Naidoo, A., Wedd, D.J., and Gatehouse, D.G. (1990). Comparison of Salmonella typhimurium TA102 with Escherichia coli WP2 tester strains. Mutagenesis 5, 285-291.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Bacterial Background Lawn Evaluation Code – Evidence for test substance toxicity to the bacteria was documented by recording the appearance of the background lawn using the following code:

T0 **Normal**, background microcolony lawn appears normal.
T1 **Slightly reduced**, background microcolony lawn is noticeably thinner.
T2 **Moderately reduced**, background lawn is markedly thinner resulting in an increase in the size of microcolonies compared to the vehicle control plate(s).
T3 **Severely reduced**, background lawn is distinguished by an extreme thinning resulting in an increase in the size of the microcolonies compared to the vehicle control plate(s). Microcolonies may be seen readily by the unaided eye and are greatly enlarged relative to controls.
T4 **Absent**, plate(s) are distinguished by a complete lack of any microcolony lawn over a majority of the area of the plate(s).

Test Substance Precipitation Code – Formation of a precipitate by the test substance was documented using the following code:

P0 **No precipitate,** no precipitate observed.
P1 **Microscopic precipitate**, precipitate present which does not interfere with background lawn evaluation or automated colony counting.
P2 **Non-interfering precipitate,** precipitate present that is visible to the unaided eye that does not interfere with automated colony counting.
P3 **Interfering precipitate,** precipitate present that requires plate to be counted by hand.
P4 **Heavy interfering precipitate**, precipitate present that prevents accurate colony counting and obscures the background lawn requiring plate rejection (R).

Lost Plate Justification Code:

L0 The loss of this test substance-treated plate does not invalidate the results since the remaining plate at this dose level and the remaining treated plates are also comparable to the negative control.
L1 The loss of this vehicle control plate does not invalidate the results since the remaining vehicle control plate is consistent with the historical negative control value for this condition.
L2 The loss of this positive control plate does not invalidate the results since the remaining positive control plate is consistent with the historical positive control value for this condition.
L3 The loss of this test substance-treated plate does not invalidate the results since the remaining plate is consistent with the remaining treated plates.
L4 The loss of this test substance-treated plate does not invalidate the results since the remaining plate at this dose level is comparable to the negative control.
L5 The loss of this untreated control plate does not invalidate the results since the remaining plate at the dose level is comparable to the vehicle control and is consistent with the historical negative control value for this condition.

Table 1
Toxicity-mutation test in *Salmonella typhimurium* TA98 without S9

Strain:	TA98	Experiment No:	T-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.93×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	121	32	T0,P0	32	0
	122	32	T0,P0		
Positive Control[b]	123	215	T0,P0	212	4
	124	209	T0,P0		
33.3	125	19	T0,P0	25	8
	126	30	T0,P0		
66.7	127	20	T0,P0	24	5
	128	27	T0,P0		
100	129	27	T0,P0	25	4
	130	22	T0,P0		
333	131	14	T0,P0	21	9
	132	27	T0,P0		
667	133	22	T0,P0	25	4
	134	27	T0,P0		
1000	135	27	T0,P0	30	4
	136	33	T0,P0		
3333	137	20	T0,P0	23	4
	138	25	T0,P0		
5000	139	27	T0,P0	21	9
	140	14	T0,P0		

[a] Sterile Water

[b] 1.0 µg/plate 2-nitroflourene

Table 2
Toxicity-mutation test in *Salmonella typhimurium* TA100 without S9

Strain:	TA100	Experiment No:	T-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.87×10^9
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	141	133	T0,P0	134	1
	142	134	T0,P0		
Positive Control[b]	143	1040	T0,P0	1041	1
	144	1042	T0,P0		
33.3	145	116	T0,P0	122	8
	146	127	T0,P0		
66.7	147	119	T0,P0	120	1
	148	121	T0,P0		
100	149	146	T0,P0	140	9
	150	133	T0,P0		
333	151	113	T0,P0	111	4
	152	108	T0,P0		
667	153	128	T0,P0	122	8
	154	116	T0,P0		
1000	155	110	T0,P0	113	4
	156	116	T0,P0		
3333	157	85	T0,P0	107	31
	158	129	T0,P0		
5000	159	133	T0,P0	123	14
	160	113	T0,P0		

[a] Sterile Water

[b] 2.0 µg/plate sodium azide

Table 3
Toxicity-mutation test in *Salmonella typhimurium* TA1535 without S9

Strain:	TA1535	Experiment No:	T-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.90×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	161	9	T0,P0	9	0
	162	9	T0,P0		
Positive Control[b]	163	669	T0,P0	761	130
	164	853	T0,P0		
33.3	165	10	T0,P0	12	3
	166	14	T0,P0		
66.7	167	6	T0,P0	8	3
	168	10	T0,P0		
100	169	4	T0,P0	6	3
	170	8	T0,P0		
333	171	4	T0,P0	9	7
	172	14	T0,P0		
667	173	13	T0,P0	12	2
	174	10	T0,P0		
1000	175	8	T0,P0	6	4
	176	3	T0,P0		
3333	177	10	T0,P0	7	5
	178	3	T0,P0		
5000	179	9	T0,P0	10	1
	180	11	T0,P0		

[a] Sterile Water

[b] 2.0 µg/plate sodium azide

Table 4
Toxicity-mutation test in *Salmonella typhimurium* TA1537 without S9

Strain:	TA1537	Experiment No:	T-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.75×10^9
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	181	5	T0,P0	10	6
	182	14	T0,P0		
Positive Control[b]	183	1696	T0,P0	1661	49
	184	1626	T0,P0		
33.3	185	5	T0,P0	5	0
	186	5	T0,P0		
66.7	187	6	T0,P0	7	1
	188	8	T0,P0		
100	189	5	T0,P0	4	1
	190	3	T0,P0		
333	191	6	T0,P0	5	2
	192	3	T0,P0		
667	193	14	T0,P0	10	6
	194	5	T0,P0		
1000	195	6	T0,P0	6	1
	196	5	T0,P0		
3333	197	4	T0,P0	5	1
	198	5	T0,P0		
5000	199	1	T0,P0	3	3
	200	5	T0,P0		

[a] Sterile Water

[b] 2.0 µg/plate ICR-191

Table 5
Toxicity-mutation test in *Escherichia coli* WP2*uvr*A without S9

Strain:	WP2*uvr*A	Experiment No:	T-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	1.21×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	101	38	T0,P0	40	3
	102	42	T0,P0		
Positive Control[b]	103	591	T0,P0	594	4
	104	596	T0,P0		
33.3	105	51	T0,P0	49	3
	106	47	T0,P0		
66.7	107	34	T0,P0	35	1
	108	35	T0,P0		
100	109	28	T0,P0	30	3
	110	32	T0,P0		
333	111	28	T0,P0	33	7
	112	38	T0,P0		
667	113	32	T0,P0	33	1
	114	34	T0,P0		
1000	115	35	T0,P0	39	6
	116	43	T0,P0		
3333	117	32	T0,P0	38	8
	118	43	T0,P0		
5000	119	42	T0,P0	37	7
	120	32	T0,P0		

[a] Sterile Water

[b] 1.0 µg/plate 4-nitroquinoline-N-oxide

Table 6
Toxicity-mutation test in *Salmonella typhimurium* TA98 with S9

Strain:	TA98	Experiment No:	T-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.93×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	21	22	T0,P0	29	9
	22	35	T0,P0		
Positive Control[b]	23	521	T0,P0	491	42
	24	461	T0,P0		
33.3	25	32	T0,P0	31	2
	26	29	T0,P0		
66.7	27	32	T0,P0	28	6
	28	24	T0,P0		
100	29	27	T0,P0	22	8
	30	16	T0,P0		
333	31	35	T0,P0	34	2
	32	32	T0,P0		
667	33	28	T0,P0	26	4
	34	23	T0,P0		
1000	35	23	T0,P0	28	6
	36	32	T0,P0		
3333	37	32	T0,P0	30	3
	38	28	T0,P0		
5000	39	28	T0,P0	36	11
	40	43	T0,P0		

[a] Sterile Water

[b] 2.5 µg/plate benzo(a)pyrene

Table 7
Toxicity-mutation test in *Salmonella typhimurium* TA100 with S9

Strain:	TA100	Experiment No:	T-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.87×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	41	163	T0,P0	144	27
	42	125	T0,P0		
Positive Control[b]	43	1035	T0,P0	1399	514
	44	1762	T0,P0		
33.3	45	166	T0,P0	153	19
	46	139	T0,P0		
66.7	47	144	T0,P0	142	3
	48	140	T0,P0		
100	49	154	T0,P0	137	25
	50	119	T0,P0		
333	51	147	T0,P0	142	7
	52	137	T0,P0		
667	53	124	T0,P0	133	13
	54	142	T0,P0		
1000	55	125	T0,P0	138	18
	56	151	T0,P0		
3333	57	120	T0,P0	134	19
	58	147	T0,P0		
5000	59	151	T0,P0	140	16
	60	129	T0,P0		

[a] Sterile Water

[b] 2.5 µg/plate 2-aminoanthracene

Table 8
Toxicity-mutation test in *Salmonella typhimurium* TA1535 with S9

Strain:	TA1535	Experiment No:	T-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.90×10^9
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	61	20	T0,P0	15	7
	62	10	T0,P0		
Positive Control[b]	63	144	T0,P0	139	8
	64	133	T0,P0		
33.3	65	11	T0,P0	13	3
	66	15	T0,P0		
66.7	67	15	T0,P0	10	7
	68	5	T0,P0		
100	69	16	T0,P0	13	5
	70	9	T0,P0		
333	71	5	T0,P0	12	10
	72	19	T0,P0		
667	73	20	T0,P0	15	8
	74	9	T0,P0		
1000	75	13	T0,P0	11	4
	76	8	T0,P0		
3333	77	14	T0,P0	12	3
	78	10	T0,P0		
5000	79	8	T0,P0	9	1
	80	10	T0,P0		

[a] Sterile Water

[b] 2.5 µg/plate 2-aminoanthracene

Table 9
Toxicity-mutation test in *Salmonella typhimurium* TA1537 with S9

Strain:	TA1537	Experiment No:	T-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.75×10^9
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	81	9	T0,P0	8	2
	82	6	T0,P0		
Positive Control[b]	83	120	T0,P0	123	4
	84	125	T0,P0		
33.3	85	10	T0,P0	8	4
	86	5	T0,P0		
66.7	87	11	T0,P0	9	4
	88	6	T0,P0		
100	89	9	T0,P0	9	1
	90	8	T0,P0		
333	91	11	T0,P0	11	1
	92	10	T0,P0		
667	93	14	T0,P0	14	0
	94	14	T0,P0		
1000	95	8	T0,P0	8	0
	96	8	T0,P0		
3333	97	13	T0,P0	9	6
	98	4	T0,P0		
5000	99	8	T0,P0	11	4
	100	13	T0,P0		

[a] Sterile Water

[b] 2.5 µg/plate 2-aminoanthracene

Table 10
Toxicity-mutation test in *Escherichia coli* WP2*uvr*A with S9

Strain:	WP2*uvr*A	Experiment No:	T-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	1.21×10^{9}
Plating Aliquot:	100 µL	Date Plated:	27-Mar-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	1	34	T0,P0	36	2
	2	37	T0,P0		
Positive Control[b]	3	411	T0,P0	373	54
	4	334	T0,P0		
33.3	5	30	T0,P0	29	2
	6	27	T0,P0		
66.7	7	43	T0,P0	44	1
	8	44	T0,P0		
100	9	40	T0,P0	38	4
	10	35	T0,P0		
333	11	52	T0,P0	46	8
	12	40	T0,P0		
667	13	35	T0,P0	42	9
	14	48	T0,P0		
1000	15	28	T0,P0	36	11
	16	44	T0,P0		
3333	17	34	T0,P0	41	9
	18	47	T0,P0		
5000	19	-	L0	32	-
	20	32	T0,P0		

[a] Sterile Water

[b] 25 µg/plate 2-aminoanthracene

Table 11
Mutagenicity test in *Salmonella typhimurium* TA98 without S9

Strain:	TA98	Experiment No:	E-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.91×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	127	18	T0P0	20	6
	128	15	T0P0		
	129	27	T0P0		
Positive Control[b]	130	196	T0P0	170	30
	131	137	T0P0		
	132	178	T0P0		
333	133	18	T0P0	23	5
	134	23	T0P0		
	135	28	T0P0		
667	136	19	T0P0	21	2
	137	20	T0P0		
	138	23	T0P0		
1000	139	22	T0P0	24	3
	140	22	T0P0		
	141	27	T0P0		
3333	142	24	T0P0	26	3
	143	29	T0P0		
	144	24	T0P0		
5000	145	18	T0P0	25	8
	146	34	T0P0		
	147	24	T0P0		

[a] sterile water

[b] 1.0 µg/plate 2-nitroflourene

Table 12
Mutagenicity test in *Salmonella typhimurium* TA100 without S9

Strain:	TA100	Experiment No:	E-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.91×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	148	123	T0P0	121	6
	149	114	T0P0		
	150	125	T0P0		
Positive Control[b]	151	1045	T0P0	1035	39
	152	1068	T0P0		
	153	992	T0P0		
333	154	121	T0P0	125	6
	155	123	T0P0		
	156	132	T0P0		
667	157	109	T0P0	109	9
	158	101	T0P0		
	159	118	T0P0		
1000	160	103	T0P0	112	11
	161	109	T0P0		
	162	125	T0P0		
3333	163	146	T0P0	130	16
	164	114	T0P0		
	165	129	T0P0		
5000	166	125	T0P0	131	5
	167	135	T0P0		
	168	133	T0P0		

[a] sterile water

[b] 2.0 µg/plate sodium azide

Table 13
Mutagenicity test in *Salmonella typhimurium* TA1535 without S9

Strain:	TA1535	Experiment No:	E-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.99×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	169	8	T0P0	13	4
	170	15	T0P0		
	171	15	T0P0		
Positive Control[b]	172	825	T0P0	810	29
	173	828	T0P0		
	174	777	T0P0		
333	175	14	T0P0	15	5
	176	11	T0P0		
	177	20	T0P0		
667	178	13	T0P0	10	3
	179	9	T0P0		
	180	8	T0P0		
1000	181	15	T0P0	20	7
	182	28	T0P0		
	183	16	T0P0		
3333	184	11	T0P0	13	5
	185	9	T0P0		
	186	19	T0P0		
5000	187	11	T0P0	15	5
	188	14	T0P0		
	189	20	T0P0		

[a] sterile water

[b] 2.0 µg/plate sodium azide

Table 14
Mutagenicity test in *Salmonella typhimurium* TA1537 without S9

Strain:	TA1537	Experiment No:	E-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	0.84×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	190	10	T0P0	11	5
	191	16	T0P0		
	192	6	T0P0		
Positive Control[b]	193	1516	T0P0	1998	422
	194	2177	T0P0		
	195	2301	T0P0		
333	196	8	T0P0	12	4
	197	15	T0P0		
	198	14	T0P0		
667	199	9	T0P0	7	3
	200	4	T0P0		
	201	9	T0P0		
1000	202	5	T0P0	7	3
	203	6	T0P0		
	204	10	T0P0		
3333	205	8	T0P0	8	2
	206	10	T0P0		
	207	6	T0P0		
5000	208	9	T0P0	13	5
	209	-	L0		
	210	16	T0P0		

[a] sterile water

[b] 2.0 µg/plate ICR-191

Table 15
Mutagenicity test in *Escherichia coli* WP2*uvr*A without S9

Strain:	WP2*uvr*A	Experiment No:	E-1
Rat Liver S9:	Absent	Cell Titer (cells/mL):	1.11×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	106	42	T0P0	37	6
	107	40	T0P0		
	108	30	T0P0		
Positive Control[b]	109	691	T0P0	670	38
	110	626	T0P0		
	111	692	T0P0		
333	112	37	T0P0	38	8
	113	46	T0P0		
	114	30	T0P0		
667	115	37	T0P0	43	6
	116	43	T0P0		
	117	48	T0P0		
1000	118	43	T0P0	34	9
	119	35	T0P0		
	120	25	T0P0		
3333	121	24	T0P0	36	11
	122	46	T0P0		
	123	37	T0P0		
5000	124	38	T0P0	40	3
	125	40	T0P0		
	126	43	T0P0		

[a] sterile water

[b] 1.0 µg/plate 4-nitroquinoline-N-oxide

Table 16
Mutagenicity test in *Salmonella typhimurium* TA98 with S9

Strain:	TA98	Experiment No:	E-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.91×10^{9}
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	22	25	T0P0	29	4
	23	32	T0P0		
	24	29	T0P0		
Positive Control[b]	25	275	T0P0	440	151
	26	572	T0P0		
	27	473	T0P0		
333	28	30	T0P0	39	12
	29	-	L0		
	30	47	T0P0		
667	31	30	T0P0	38	7
	32	39	T0P0		
	33	44	T0P0		
1000	34	38	T0P0	35	3
	35	34	T0P0		
	36	33	T0P0		
3333	37	34	T0P0	33	6
	38	39	T0P0		
	39	27	T0P0		
5000	40	39	T0P0	29	8
	41	25	T0P0		
	42	24	T0P0		

[a] sterile water

[b] 2.5 µg/plate benzo(a)pyrene

Table 17
Mutagenicity test in *Salmonella typhimurium* TA100 with S9

Strain:	TA100	Experiment No:	E-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.91×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	43	135	T0P0	137	19
	44	120	T0P0		
	45	157	T0P0		
Positive Control[b]	46	2336	T0P0	2501	242
	47	2779	T0P0		
	48	2388	T0P0		
333	49	144	T0P0	160	15
	50	173	T0P0		
	51	162	T0P0		
667	52	158	T0P0	158	1
	53	158	T0P0		
	54	157	T0P0		
1000	55	165	T0P0	144	21
	56	124	T0P0		
	57	143	T0P0		
3333	58	171	T0P0	155	16
	59	139	T0P0		
	60	154	T0P0		
5000	61	167	T0P0	142	22
	62	135	T0P0		
	63	125	T0P0		

[a] sterile water

[b] 2.5 µg/plate 2-aminoanthracene

Table 18
Mutagenicity test in *Salmonella typhimurium* TA1535 with S9

Strain:	TA1535	Experiment No:	E-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.99×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	64	14	T0P0	11	3
	65	10	T0P0		
	66	8	T0P0		
Positive Control[b]	67	156	T0P0	169	13
	68	181	T0P0		
	69	171	T0P0		
333	70	9	T0P0	16	7
	71	22	T0P0		
	72	16	T0P0		
667	73	11	T0P0	14	4
	74	13	T0P0		
	75	18	T0P0		
1000	76	18	T0P0	18	2
	77	20	T0P0		
	78	16	T0P0		
3333	79	16	T0P0	17	1
	80	16	T0P0		
	81	18	T0P0		
5000	82	13	T0P0	10	3
	83	10	T0P0		
	84	8	T0P0		

[a] sterile water

[b] 2.5 µg/plate 2-aminoanthracene

Table 19
Mutagenicity test in *Salmonella typhimurium* TA1537 with S9

Strain:	TA1537	Experiment No:	E-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	0.84×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	85	4	T0P0	11	8
	86	19	T0P0		
	87	11	T0P0		
Positive Control[b]	88	238	T0P0	213	25
	89	189	T0P0		
	90	211	T0P0		
333	91	28	T0P0	20	11
	92	8	T0P0		
	93	24	T0P0		
667	94	15	T0P0	16	3
	95	20	T0P0		
	96	14	T0P0		
1000	97	9	T0P0	11	3
	98	15	T0P0		
	99	10	T0P0		
3333	100	15	T0P0	13	2
	101	11	T0P0		
	102	13	T0P0		
5000	103	14	T0P0	11	3
	104	8	T0P0		
	105	10	T0P0		

[a] sterile water

[b] 2.5 µg/plate 2-aminoanthracene

Table 20
Mutagenicity test in *Escherichia coli* WP2*uvr*A with S9

Strain:	WP2*uvr*A	Experiment No:	E-1
Rat Liver S9:	Present	Cell Titer (cells/mL):	1.11×10^9
Plating Aliquot:	100 µL	Date Plated:	3-Apr-07

Dose (µg/plate)	Plate Number	Revertants Per Plate	Background Code	Mean	SD
Vehicle[a]	1	37	T0P0	39	5
	2	44	T0P0		
	3	35	T0P0		
Positive Control[b]	4	256	T0P0	292	56
	5	263	T0P0		
	6	357	T0P0		
333	7	49	T0P0	42	8
	8	43	T0P0		
	9	34	T0P0		
667	10	37	T0P0	36	3
	11	39	T0P0		
	12	33	T0P0		
1000	13	44	T0P0	43	2
	14	44	T0P0		
	15	40	T0P0		
3333	16	43	T0P0	41	13
	17	27	T0P0		
	18	53	T0P0		
5000	19	46	T0P0	43	9
	20	49	T0P0		
	21	33	T0P0		

[a] sterile water

[b] 25 µg/plate 2-aminoanthracene

Table 21
Summary of the toxicity-mutation test without rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	32	0	134	1	9	0	10	6	40	3
positive control	212	4	1041	1	761	130	1661	49	594	4
33.3	25	8	122	8	12	3	5	0	49	3
66.7	24	5	120	1	8	3	7	1	35	1
100	25	4	140	9	6	3	4	1	30	3
333	21	9	111	4	9	7	5	2	33	7
667	25	4	122	8	12	2	10	6	33	1
1000	30	4	113	4	6	4	6	1	39	6
3333	23	4	107	31	7	5	5	1	38	8
5000	21	9	123	14	10	1	3	3	37	7

Experiment No: T-1
Plate Aliquot: 100 µL

Table 22
Summary of the toxicity-mutation test with rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	29	9	144	27	15	7	8	2	36	2
positive control	491	42	1399	514	139	8	123	4	373	54
33.3	31	2	153	19	13	3	8	4	29	2
66.7	28	6	142	3	10	7	9	4	44	1
100	22	8	137	25	13	5	9	1	38	4
333	34	2	142	7	12	10	11	1	46	8
667	26	4	133	13	15	8	14	0	42	9
1000	28	6	138	18	11	4	8	0	36	11
3333	30	3	134	19	12	3	9	6	41	9
5000	36	11	140	16	9	1	11	4	32[a]	-

Experiment No: T-1
Plate Aliquot: 100 µL
a Count based on 1 plate.

Table 23
Summary of the mutagenicity test without rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	20	6	121	6	13	4	11	5	37	6
positive control	170	30	1035	39	810	29	1998	422	670	38
333	23	5	125	6	15	5	12	4	38	8
667	21	2	109	9	10	3	7	3	43	6
1000	24	3	112	11	20	7	7	3	34	9
3333	26	3	130	16	13	5	8	2	36	11
5000	25	8	131	5	15	5	13[a]	5	40	3

Experiment No: E-1
Plate Aliquot: 100 µL
a Count based on 2 plates.

Table 24
Summary of the mutagenicity test with rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	29	4	137	19	11	3	11	8	39	5
positive control	440	151	2501	242	169	13	213	25	292	56
333	39[a]	12	160	15	16	7	20	11	42	8
667	38	7	158	1	14	4	16	3	36	3
1000	35	3	144	21	18	2	11	3	43	2
3333	33	6	155	16	17	1	13	2	41	13
5000	29	8	142	22	10	3	11	3	43	9

Experiment No: E-1
Plate Aliquot: 100 µL
a Count based on 2 plates.

APPENDICES

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28072
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	82.6%
Other Components	Water – 13.9% Ammonium (excess) – 3.5%
Date of Analysis	July 19, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-23285
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Peter A. Bloxham, Ph.D.
Analyst's Name

Analyst's signature

23-JULY-2007
Date

Revision #1
July 20, 2007

Appendix B
Historical Control Data

Historical Control Data[a]

Tester Strain	Control [Positive Control][b]	Exogenous Activation System	Mean	SD	Range Minimum	-	Range Maximum
TA98	Negative	Absent	20	7	4	-	50
	Negative	Present	29	8	10	-	58
	Positive [2NF-1]	Absent	187	54	39	-	394
	Positive [BAP-2.5]	Present	405	86	99	-	689
TA100	Negative	Absent	110	23	54	-	253
	Negative	Present	130	23	63	-	242
	Positive [SA-2]	Absent	1078	188	535	-	1803
	Positive [2AA-2.5]	Present	2478	681	566	-	4500
TA1535	Negative	Absent	13	6	3	-	44
	Negative	Present	12	4	3	-	34
	Positive [SA-2]	Absent	902	199	329	-	1549
	Positive [2AA-2.5]	Present	216	56	96	-	405
TA1537	Negative	Absent	7	3	1	-	19
	Negative	Present	9	4	1	-	29
	Positive [ICR 191-2]	Absent	1677	500	552	-	3470
	Positive [2AA-2.5]	Present	168	76	40	-	484
WP2*uvr*A	Negative	Absent	33	9	10	-	65
	Negative	Present	39	10	11	-	69
	Positive [4NQO-1]	Absent	762	213	273	-	1259
	Positive [2AA-25]	Present	441	115	167	-	848

a Historical data for tester strains used in the reported study. Data are based on studies reported since 2004. Data include all control solvents or diluents, and metabolic activation systems based on Aroclor®-induced rat liver S9.

b Abbreviations for positive controls: SA (sodium azide); 2AA (2-aminoanthracene); 2NF (2-nitrofluorene); ICR 191 (ICR 191 Acridine mutagen); 4NQO (4-nitroquinoline-N-oxide); BAP (benzo[a]pyrene). The number following abbreviation is the microgram (µg) amount per plate or vial used for the positive control.

c SD = standard deviation